

## Acute Coronary Syndromes

# Evidence of Prolonged Inflammation in Unstable Angina and Non-Q wave Myocardial Infarction

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| <b>OBJECTIVES</b>  | This study was designed to document the inflammatory response up to one year after acute presentation with unstable angina (UA) and non-Q wave infarction (NQMI) as reflected by the expression of soluble cell adhesion molecules (CAMs).  |
| <b>BACKGROUND</b>  | Coronary plaque inflammation is a key component in the pathogenesis of acute coronary syndromes. Cell adhesion molecules are critical mediators of the inflammatory process. Soluble forms of these molecules are detectable in serum and are elevated acutely in patients with UA and NQMI.  |
| <b>METHODS</b>     | Patients presenting with UA and NQMI had serum samples taken at presentation and then after three, six and 12 months. A control group of similar age and gender distribution was used for comparison. Levels of soluble inter-cellular adhesion molecule-1, vascular cell adhesion molecule-1, endothelial-selectin and platelet-selectin were measured using an ELISA technique.                 |
| <b>RESULTS</b>     | We studied 91 patients (M/F = 73/18, mean age $62 \pm 11$ years, 56 UA and 35 NQMI) and 24 controls (M/F = 18/6, mean age $56 \pm 12$ years). Levels of all four soluble CAMs were significantly elevated in both UA and NQMI patients at presentation, three and six months in comparison with controls. Levels in UA and NQMI groups fell between six and 12 months after initial presentation. |
| <b>CONCLUSIONS</b> | The results suggest that the inflammatory stimulus triggering expression of CAMs is sustained for up to six months after presentation with either UA or NQMI and then returns toward control values over the following six months. (J Am Coll Cardiol 2000;36:1210-6)<br>© 2000 by the American College of Cardiology   |

The pathophysiological mechanisms responsible for unstable angina (UA) and non-Q wave myocardial infarction (NQMI) are believed to involve an acute inflammatory stimulus that contributes to coronary plaque disruption and subsequent platelet aggregation and vessel thrombosis (1-7). The hypothesis of an acute inflammatory reaction playing a role in destabilizing the fibrous tissue cap of the atherosclerotic plaque is supported by the finding that inflammatory cells, predominantly macrophages, are present at the site of plaque erosion or rupture (8). Further evidence in support of the inflammatory hypothesis comes from the finding of activated leukocytes in the coronary and systemic circulation (9), the release of thromboxanes and leukotrienes related to the occurrence of ischemic chest pain (10) and elevated levels of the acute phase reactants C-reactive protein (CRP) and serum amyloid A protein (SSA) in patients with acute coronary syndromes (11,12). The rise in CRP in patients with UA occurs independently of myocardial cell injury or death (13,14).

The inflammatory response is associated with the generation of cytokines and other inflammatory mediators that

induce increased expression of cellular adhesion molecules (CAMs) (15,16). These CAMs play a critical role in the adhesion and transendothelial migration of leukocytes from the blood to the arterial intima (17). Intercellular adhesion molecule-1 (ICAM-1) is expressed on both resting and activated endothelial cells and promotes adhesion of neutrophils, monocytes and lymphocytes (18). Vascular cell adhesion molecule-1 (VCAM-1) binds monocytes and T-lymphocytes to activated endothelial cells (16). Endothelial selectin (E-selectin) is expressed by activated endothelial cells and binds to mucin like cell adhesion molecules on the neutrophil surface membrane, thus initiating neutrophil extravasation (19). Platelet selectin (P-selectin) is similarly expressed by activated endothelial cells and platelets and mediates leukocyte adhesion to platelets and endothelial cells during inflammation, thrombosis and atherosclerosis (16,20).

The extracellular portion of these adhesion proteins can be cleaved by proteolytic enzymes to form a circulating molecules that can be detected in serum and are referred to as the soluble cell adhesion molecules (sCAMs) (21,22). Alternative splicing of the messenger RNA encoding for P-selectin can generate a soluble form of the protein that can be detected in serum (23). Levels of sICAM-1, sVCAM-1 and sE-selectin are elevated in patients with UA and NQMI throughout the first 72 h of acute presentation (24). Soluble P-selectin levels are elevated in patients with

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# Abbreviations and Acronyms

|             |   |
|-------------|---|
| ANOVA       | = analysis of variance                      |
| CAM         | = cellular adhesion molecule                |
| CRP         | = C-reactive protein                        |
| sE-selectin | = soluble endothelial selectin              |
| sICAM-1     | = soluble intercellular adhesion molecule-1 |
| MI          | = myocardial infarction                     |
| NQMI        | = non-Q wave myocardial infarction          |
| sP-selectin | = soluble platelet selectin                 |
| SSA         | = serum amyloid A protein                   |
| UA          | = unstable angina                           |
| sVCAM-1     | = soluble vascular cell adhesion molecule-1 |

UA in comparison with patients with stable angina and controls (25). The pathological significance of these soluble forms may be underestimated as they can interfere with leukocyte-endothelial interactions in vivo as has already been demonstrated in vitro (26). Alteration in concentrations of these soluble adhesion molecules may relate to activation or damage of various cell types including platelets and endothelial cells. There is recent evidence that platelet activation and inflammation continues for many weeks after the acute ischemic event in patients with acute coronary syndromes (27,28). Thus, the primary aim of this study was to characterize the temporal expression of four soluble CAMs: sICAM-1, sVCAM-1, sE-selectin and sP-selectin up to 12 months after acute presentation with either UA or NQMI.

## METHODS

**Study population.** Study approval was granted by the institutional ethics review committee, and written informed consent was obtained from all patients. Patients presenting acutely with either UA or NQMI were recruited into this study. Unstable angina was defined according to the Braunwald classification as angina at rest within the preceding 24 h, with either ST segment depression ( $>0.1\text{mV}$ ) or T-wave inversion in two or more contiguous leads on the presenting electrocardiogram and serum creatine kinase levels within the normal range (29). Patients in the NQMI group had similar diagnostic criteria along with an elevation in creatine kinase.

Our group has recently reported that successful thrombolysis causes a significant elevation in levels of sCAMs, and, thus, we decided not to enroll patients who were treated with thrombolysis but instead concentrate on patients with UA and NQMI who receive similar treatment and have comparable clinical outcomes (30). Study patients had an initial venous sample taken before commencing antithrombotic therapy with heparin. The study patients were monitored in the coronary care unit. Decisions regarding the duration of treatment with antithrombotics and the need for coronary revascularization were made by the attending cardiologist. Patients with an active acute or chronic inflammatory illness, those taking immunosuppres-

**Table 1.** Clinical Characteristics

|   | UA<br>(n = 56) | NQMI<br>(n = 35) | Controls<br>(n = 24) |
|---|----------------|------------------|----------------------|
| Age (yrs)   | 60 $\pm$ 10    | 64 $\pm$ 11*     | 56 $\pm$ 11          |
| Male gender (%)   | 43 (77)        | 30 (81)          | 18 (75)              |
| Diabetes mellitus (%)   | 11 (20)        | 4 (11)           | 0                    |
| Hypercholesterolemia (%)<br>(total cholesterol $>220$<br>mg/dl) | 30 (54)        | 14 (40)          | 2 (8)                |
| Smokers (%)   | 28 (50)        | 14 (40)          | 3 (12)               |
| Hypertension (%)  | 23 (41)        | 11 (31)          | 1 (4)                |
| Medications (%)   |                |                  |                      |
| Aspirin   | 49 (88)        | 32 (91)          | 1 (4)                |
| Beta-blocker  | 33 (59)        | 21 (60)          | 0                    |
| Ca blocker  | 31 (55)*       | 9 (26)           | 0                    |
| Nitrate   | 27 (48)        | 15 (43)          | 0                    |
| ACE inhibitor   | 17 (30)        | 11 (31)          | 1 (4)                |
| HMG CoA   | 27 (48)        | 14 (40)          | 3 (12)               |

\*p < 0.05 NQMI vs. UA.

ACE = angiotensin-converting enzyme; Ca = calcium; NQMI = non-Q wave myocardial infarction; UA = unstable angina.

sive therapy or those who had taken recent antibiotic therapy, were excluded from the study. Twenty four volunteers in the same age distribution and similar male/female ratio acted as a healthy control population.

**Adhesion molecule estimation.** Patients and volunteers had peripheral venous blood samples taken at presentation and after 3, 6 and 12 months. These samples were centrifuged and serum was stored at  $-70^{\circ}\text{C}$ . Levels of sICAM-1, sVCAM-1, sE-selectin and sP-selectin were measured in stored sera using enzyme linked immunosorbent assay kits supplied by R&D Systems (Abingdon, United Kingdom). The assay is standardized against a purified form of recombinant ICAM-1, VCAM-1, E-selectin and P-selectin. Repeat measurement of 33% of the samples was performed during each assay to determine the variability of the measurement.

**Statistical analysis.** Soluble adhesion molecule levels were measured in ng/ml and expressed as mean  $\pm$  SEM for the UA, NQMI and control groups at each sampling time point. A two-way analysis of variance (ANOVA) for repeated measures was performed to analyze differences in levels of soluble CAMs between the UA, NQMI and control groups at different time points. Single factor ANOVA was used to investigate differences in sCAM levels among the three groups at each measured time point. A 5% level of significance was used for the two-way ANOVA and a 1% level of significance for the single factor ANOVA. Details of the study and control populations are expressed as mean  $\pm$  standard deviation. A multiple regression analysis model was used to assess the effect of cardiovascular drug therapy on the levels of soluble CAMs. The levels sICAM, sVCAM-1, sE-selectin and sP-selectin were designated as dependent variables and the cardiovascular drugs outlined in Table 1. were added to the model in a forward stepping manner. Statistical analysis was performed with an SPSS software package.

**Table 2.** Major Adverse Cardiovascular Events and Revascularization

|                       | UA<br>(n = 56) | NQMI<br>(n = 35) |
|-----------------------|----------------|------------------|
| MACE (%)              | 17 (30)        | 10 (29)          |
| Recurrent UA          | 12 (21)        | 2 (6)            |
| Nonfatal MI           | 3 (5)          | 4 (11)           |
| Cardiovascular death  | 2 (4)          | 4 (11)           |
| Revascularization (%) | 25 (44)        | 13 (35)          |
| PCI                   | 17 (30)        | 10 (29)          |
| CABG                  | 8 (14)         | 3 (8)            |

CABG = coronary artery bypass grafting; MACE = major adverse cardiovascular events; MI = myocardial infarction; NQMI = non-Q wave myocardial infarction; PCI = percutaneous coronary intervention; UA = unstable angina.

## RESULTS

Ninety-one patients were enrolled into the study (men/women = 73/18), 56 with UA and 35 with NQMI. The mean age of patients with NQMI was older than those with UA ( $64 \pm 11$  years vs.  $60 \pm 10$  years,  $p < 0.05$ ). The cardiovascular risk factor profiles in both UA and NQMI groups were similar (Table 1). There was a higher percentage of patients in the UA group treated with calcium antagonists (55% vs. 26%,  $p < 0.01$ ); otherwise, both ischemic groups received similar medical treatment. The major adverse cardiovascular event rate for the duration of the study was 30% in the UA group and 29% in the NQMI group (Table 2). There were five deaths during the acute admission, and one patient was lost to follow-up; thus, there were 85 patients reviewed three months after discharge. One further patient died between the three and six month follow-up visits, and, therefore, there were 84 patients studied at the six- and 12-month reviews.

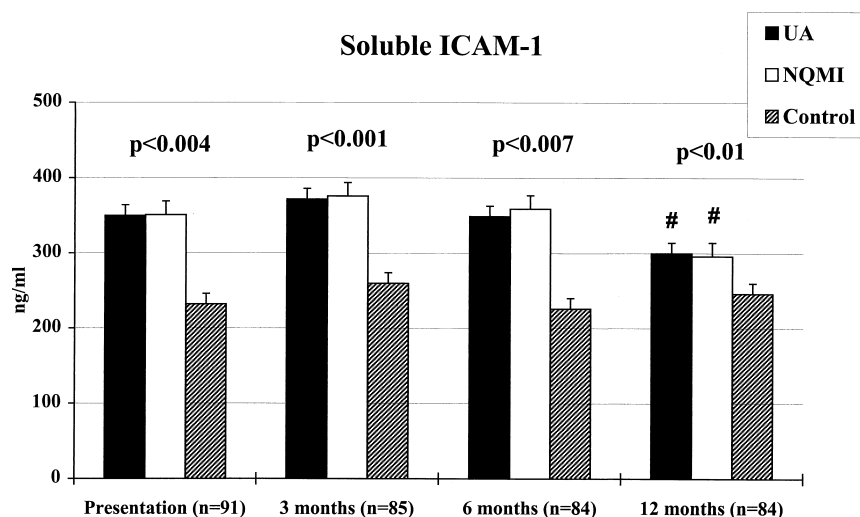
A total of 37 (66%) patients in the UA group and 22 (63%) of patients in the NQMI group underwent diagnostic coronary angiography confirming the presence of coronary artery disease in all cases. The coronary revascularization

rate was 44% in the UA group and 37% in those with NQMI (Table 2). The control group consisted of 24 healthy volunteers (male/female = 18/6, mean age  $56 \pm 11$  years) who were hospital-based employees with no documented history nor any symptoms of vascular disease. Among the control group 3 of 24 (12.5%) were smokers, and one had a history of hypertension.

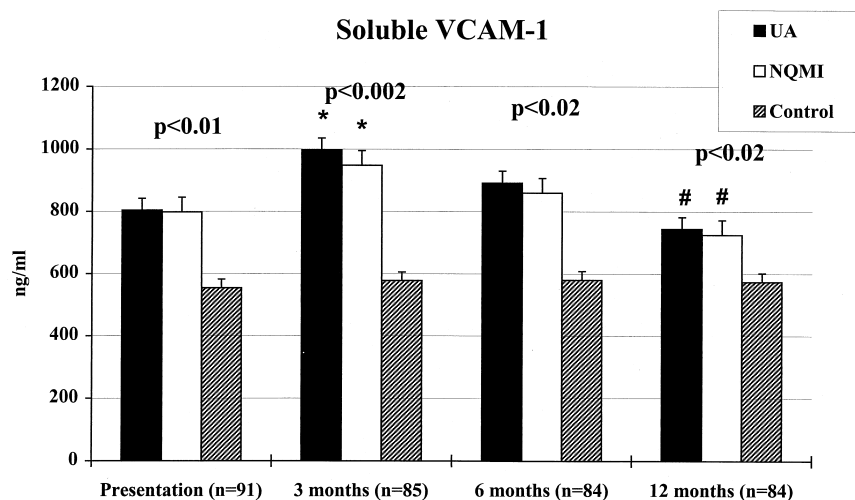
**sICAM-1.** There was no significant difference in the levels of sICAM-1 at the various measured time points between the UA and NQMI groups; however, the levels were elevated at all times in comparison with the control group. This difference in levels of sICAM-1 between the two study groups and the control group was statistically significant at presentation, three and six months (Fig. 1). Levels of sICAM-1 fell significantly in both the UA and the NQMI groups between 6 and 12 months:  $349 \pm 12$  ng/ml versus  $300 \pm 10$  ng/ml,  $p < 0.01$  for UA group and  $359 \pm 26$  ng/ml versus  $296 \pm 15$  ng/ml,  $p < 0.05$  for NQMI group.

**sVCAM-1.** There was no significant difference in levels of sVCAM-1 at the various time points between the UA and NQMI groups. Levels of sVCAM-1 in both UA and NQMI groups were significantly elevated at all time points in comparison with the control group (Fig. 2). Levels of sVCAM-1 in both UA and NQMI patients increased between presentation and three months:  $810 \pm 44$  ng/ml to  $1,004 \pm 56$  ng/ml,  $p < 0.02$  for the UA group and  $786 \pm 78$  ng/ml to  $930 \pm 82$  ng/ml,  $p < 0.03$  in the NQMI group. Then levels of sVCAM-1 fell significantly between six and 12 months in both UA and NQMI groups:  $892 \pm 48$  ng/ml versus  $744 \pm 34$  ng/ml,  $p < 0.03$  for the UA group and  $860 \pm 63$  ng/ml versus  $725 \pm 22$  ng/ml,  $p < 0.05$  for the NQMI group.

**sE-selectin.** The levels of sE-selectin were elevated in both ischemic groups in comparison with the control group at presentation, three and six months, respectively (Fig. 3).



**Figure 1.** Levels of sICAM-1 in ng/ml in UA, NQMI and control groups at four sampling time points. p values refer to differences in levels of soluble ICAM-1 between the three groups. #p < 0.05 for levels of sICAM-1 at 6 months versus levels at 12 months in UA and NQMI groups. ICAM-1 = intercellular adhesion molecule-1; NQMI = non-Q wave myocardial infarction; UA = unstable angina.



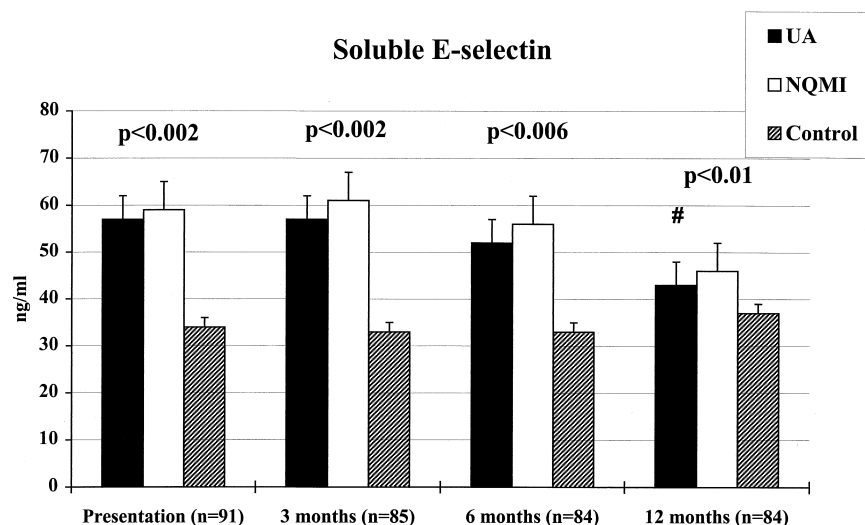
**Figure 2.** Levels of sVCAM-1 in ng/ml in UA, NQMI and control groups at four sampling time points. p values refer to differences in levels of sVCAM-1 between the three groups. \*p < 0.05 for levels of sVCAM-1 in both UA and NQMI groups between presentation and three months. #p < 0.05 for levels of sVCAM-1 at 6 months versus levels at 12 months in UA and NQMI groups. NQMI = non-Q-wave myocardial infarction; UA = unstable angina; VCAM-1 = vascular cell adhesion molecule.

There was no significant difference in levels of sE-selectin between the UA and NQMI groups throughout the 12 months of observation. There was a fall in levels of sE-selectin in both study groups between the 6 and 12 month sampling period:  $52 \pm 3$  ng/ml versus  $42 \pm 3$  ng/ml, p < 0.01 for UA and  $56 \pm 4$  ng/ml versus  $46 \pm 4$  ng/ml, p < 0.1 for NQMI.

**sP-selectin.** Soluble P-selectin levels were significantly elevated at all measured time points in both UA and NQMI groups in comparison with controls (Fig. 4). There was a trend for levels of sP-selectin to be higher in the patients with NQMI in comparison with those with UA; however, this difference failed to achieve statistical significance at any sampling point. Levels of sP-selectin increased between the time of presentation and three months follow-up in both

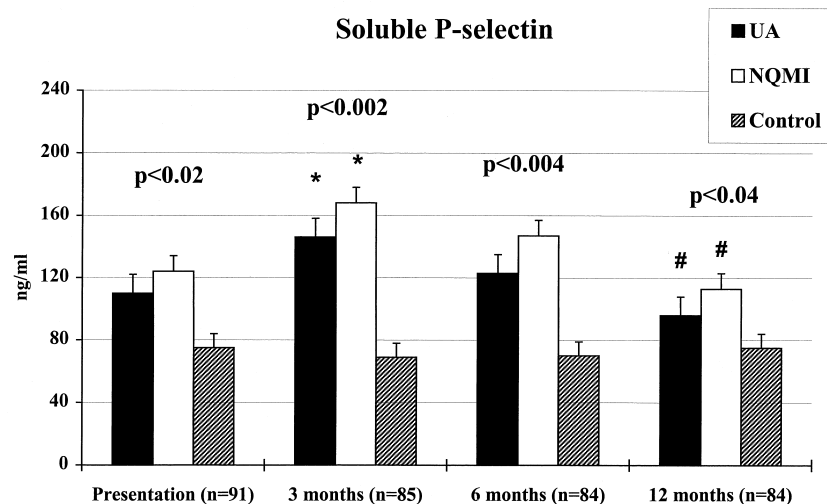
UA and NQMI groups:  $113 \pm 12$  ng/ml to  $144 \pm 13$  ng/ml, p < 0.03 in the UA group and  $120 \pm 13$  ng/ml to  $174 \pm 16$  ng/ml p < 0.02 in the NQMI group. Similarly to levels of the other soluble CAMs, sP-selectin levels fell significantly in both study groups between 6 and 12 months after initial presentation:  $123 \pm 10$  ng/ml versus  $96 \pm 9$  ng/ml, p < 0.02 for UA and  $147 \pm 12$  ng/ml versus  $113 \pm 11$  ng/ml, p < 0.02 for NQMI.

**Clinical outcome and levels of CAMs.** Levels of sVCAM-1 at presentation were significantly elevated in patients who went on to have a major adverse cardiovascular event (recurrent UA, nonfatal myocardial infarction, death) in the six months after the index event:  $979 \pm 30$  ng/ml versus  $729 \pm 22$  ng/ml, p < 0.001. There was no significant difference in the levels of sICAM-1, sE-selectin or sP-



**Figure 3.** Levels of sE-selectin in ng/ml in UA, NQMI and control groups at four sampling time points. p values refer to differences in levels of sE-selectin between the three groups. #p < 0.05 for levels of sE-selectin at 6 months versus levels at 12 months in UA and NQMI groups. E-selectin = endothelial selectin; NQMI = non-Q wave myocardial infarction; UA = unstable angina.





**Figure 4.** Levels of sP-selectin in ng/ml in UA, NQMI and control groups at four sampling time points. p values refer to differences in levels of sP-selectin between the three groups. \* $p < 0.05$  for levels of sP-selectin in both UA and NQMI groups between presentation and three months. # $p < 0.05$  for levels of sP-selectin at 6 months versus levels at 12 months in UA and NQMI groups. NQMI = non-Q wave myocardial infarction; P-selectin = platelet selectin; UA = unstable angina.

selectin between those who did and those who did not have major adverse events. There was no significant change in the levels of sCAMs in patients taking various cardiovascular medications including angiotensin-converting enzyme inhibitors or HMG-CoA reductase inhibitors over the duration of the study.

## DISCUSSION

The principal finding of this study is that the levels of sICAM-1, sVCAM-1, sE-selectin and sP-selectin were elevated in patients presenting acutely with UA and NQMI in comparison with controls, remained raised for six months after the index ischemic event and then fell over the following six months. This study included patients with both UA as well as NQMI, as similar pathogenic triggers are responsible for both conditions.

Surface ICAM-1 and VCAM-1 are critically involved with neutrophil, lymphocyte and monocyte binding and extravasation at a site of vascular injury (16). These leukocyte-endothelial cell interactions are mediated by ICAM-1 or VCAM-1 binding integrins receptors (Mac-1 and VLA-1) on the surface of these inflammatory cells (17). In vitro models have demonstrated that levels of soluble CAMs directly reflect endothelial cell surface CAM expression when cultured endothelial cells are stimulated by inflammatory cytokines (31). Levels of sICAM-1, sVCAM-1 and sE-selectin are elevated throughout the first 72 h of acute presentation in patients with UA and NQMI (24). The persistent elevation of levels of sICAM-1 and sVCAM-1 after presentation with either UA or NQMI in this study is suggestive that there is a sustained inflammatory response for up to six months in these patients, reflecting continued accumulation of inflammatory cells at the site of vascular injury.

Levels of sE-selectin and sP-selectin also remained ele-

vated for up to six months in patients presenting with the acute coronary syndromes of UA and NQMI. Endothelial-selectin is only expressed when endothelial cells are activated or stimulated (15). Platelet-selectin is released from Weibel-Palade bodies of activated endothelial cells and from alpha granules of activated platelets (32). Both E- and P-selectin are involved in binding of leukocytes to an area of activated endothelium initiating the localization of inflammatory cells at the site of vascular injury (16). Our findings suggest that there is continued activation of platelets and endothelial cells, with consequential upregulation in expression of surface E- and P-selectin and persistently elevated levels of E- and P-selectin for up to six months after the initial ischemic event. The fall in levels of soluble CAMs between 6 and 12 months may reflect waning of the inflammatory process.

Interestingly, the levels of both sVCAM-1 and sP-selectin at three months were significantly elevated in comparison with the levels at the time of presentation. These findings support the hypothesis of ongoing inflammation after clinical stabilization but also suggest that components of the inflammatory process are not maximally expressed at the time of clinical presentation.

**Inflammation in acute coronary syndromes.** There is extensive evidence of both local and systemic inflammation in acute coronary syndromes. Pathological studies have demonstrated an inflammatory process at the site of plaque rupture with a dense inflammatory cell infiltrate and increased local expression of surface CAMs (5,33,34). Activation of circulating leukocytes, release of thromboxanes and leukotrienes and increased levels of the inflammatory marker CRP provide evidence for a systemic inflammatory reaction in acute coronary syndromes (9-12). Acute coronary syndromes are characterized by persistent instability for weeks to months after the resolution of the clinical symp-

toms, which can result in recurrent episodes of UA progressing to acute myocardial infarction (MI) or death. There is recent evidence suggesting that the inflammatory process continues despite the resolution of clinical symptoms. Biasucci et al. (28) reported that serum CRP levels remain elevated at the time of discharge and at three month follow-up in up to 50% of patients who presented with Braunwald IIIB UA, and such an elevation of CRP was associated with frequent hospital readmission for recurrent instability (28). Thus, there is a potential link between recurrent ischemic episodes and persistent inflammatory stimuli. A recent paper by Ault et al. (27) reported that there is evidence of continued activation of platelets after an acute ischemic coronary event. Platelet associated P-selectin is a sensitive measure of platelet activation; this marker remained elevated for up to one month after clinical stabilization after UA or acute MI. Persistent platelet activation may be a consequence of sustained inflammatory stimuli. The authors also found a weak correlation between platelet activation parameters and levels of serum CRP. Our results strongly support the hypothesis of a persistent inflammatory response after resolution of clinical symptoms in patients with acute coronary syndromes.

**Clinical relevance.** Persistent elevation of acute phase proteins for up to three months after an ischemic cardiac event is associated with increased incidence of recurrent instability within one year (28). The findings of this study suggest that the inflammatory response remains active for up to six months after acute presentation with either UA or NQMI. We recently reported that an elevated level of sVCAM-1 at presentation in patients with acute coronary syndromes is associated with an increased risk of recurrent instability, nonfatal MI and cardiovascular death over the six months after presentation (35). These data indicate that inflammatory markers can be used to identify patients at high risk of major adverse cardiac events and are suggestive that the intensity of the inflammatory process is associated with clinical outcome in patients with acute coronary syndromes. This raises the possibility of designing specific anti-inflammatory therapies (monoclonal antibodies to CAMs) for patients at high risk of future ischemic events.

**Study limitations.** We have assumed that levels of soluble CAMs sampled from a peripheral vein directly reflect levels within the coronary circulation, at the site of the atherosclerotic plaque erosion or rupture. To resolve this issue we would have to carry out simultaneous sampling from within the coronary circulation and from a peripheral vein, which would be an unethical undertaking in a study requiring recurrent sampling over a period of 12 months. We did not address the acute effect of: 1) recurrent ischemic events or 2) coronary revascularization on the levels of sCAMs in this study but rather documented the natural time course of expression of the molecules over a 12 month period after acute presentation. The acute expression of these molecules during UA, acute MI and coronary revascularization has been previously reported (24,25,36,37). Finally we did not

use a group of patients with stable angina as a control group. Our group and others have previously reported that levels of sCAMs are elevated in patients with UA in comparison with patients with stable angina, and these findings are believed to be a consequence of the acute inflammatory process involved in the pathogenesis of acute coronary syndromes. Our aim in this study was to document the duration of this inflammatory response in patients with UA and NQMI.

**Conclusions.** The results of this study indicate that inflammatory stimuli, causing increased levels of sCAMs, persist for up to six months after an episode of UA or NQMI. The persistence of the inflammatory response and its association with clinical outcome raises the intriguing possibility of using the vascular inflammation as a novel therapeutic target in with acute coronary syndromes. Monoclonal antibodies to CAMs could represent an ideal way to block the leukocyte, endothelial cell and platelet interactions that are critical in the pathogenesis of these syndromes.

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